INTERRELATIONSHIPS OF WATER SOLUBLE VITAMINS IN RAT TISSUES

BY

LEO JOSEPH NOVAK

A. B., University of Illinois, 1935

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHYSIOLOGICAL CHEMISTRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1943

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Although rapid advances in biochemical and nutritional knowledgs of the water soluble vitamins has occurred, the etiology of nutritional alopecia still remains obscure.

The following studies on water soluble vitamin interrelationships were performed as a result of some nutritional work in which weanling albino rats were fed a basal diet containing purified cornstarch, casein and butterfat, plus salt mixture and vitamins A, D, thiamine and riboflavin. The principal symptom observed was a generalized alopecia which was so extensive as to make further studies seem profitable.

Three kinds of basal diet were prepared. "A" diet is described. "B" diet contained Crisco instead of butterfat. "C" diet contained glucose and corn oil instead of purified starch and butterfat or Crisco. The proportions of these dietary constituents resembled "regular" synthetic diets as used in rat dietary experiments.

Groups of from four to ten weanling albino rats on diet "A" received only supplements of single water soluble vitamins, while those on "B" diet ingested multiple vitamin supplements. Those on "C" diet received both single and multiple water soluble vitamin supplements (Table I).

TABLE I.

Supplement	Basal Diet	Concentration of Supplements in Micrograms per Gram of diet.*
None or thiamine, riboflavin, pyridoxine, pantothenic acid, choline, inositol or biotin	"A"	100 except biotin (1 microgm)
Thiamine plus riboflavin	"B"	10, 10
plus		
Vitamin E	"B"	10 cc. Viobin/500 gms. diet
plus		
Pyridoxine hydrochloride	"B"	15
plus		
Pantothenic acid (Ca salt)	"B"	17
plus		
Choline hydrochloride	"B"	75
plus		
Inositol	"B"	250
Thiamine, riboflavin, pyridoxine hydrochloride, calcium pantothenate, choline hydrochloride, inositol, nicotinic acid, biotin concentrate	"C"	10, 20, 10, 30, 80, 80, 40, 0.2 respectively
Pyridoxine hydrochloride	"C"	100
Choline hydrochloride	"C"	100

*Unless otherwise indicated.

Animals were fed these diets for 30 days or more. During this time, weights and symptoms were recorded. At various time intervals after 30 days, red and white cell counts were determined on some animals. The blood was obtained by tail excision. Most animals were killed at 30 days and the riboflavin, pantothenic acid,

niacin and inositol content of the kidney and liver tissue and gastro-intestinal content was determined. The hair was removed and the nitrogen to sulphur ratio was determined. Portions of the hair were also used for the determination of hair vitamin or "vitamin-like" content. A ball mill grinding technique was developed to obtain the hair in soluble form.

The microbiological bacterial water soluble vitamin assay methods were used. The technique included enzymatic digestion and ether extraction of the samples. A modified method for inositol using Saccharomyces Cerevisae "Gebruder Mäyer" as the test organism gave satisfactory results in most cases. Four vitamins were determined, namely, riboflavin, pantothenic acid, niacin and inositol.

In order to eliminate errors in the microbiological vitamin assay methods, the following substances were tested for possible interference: sodium glycocholate and taurocholate, crystalline pepsin and albumin, urobilinogen, choline, peptone and purified starch, casein, and a concentrate from extracted purified "vitamin-free" casein. It was found that under the experimental conditions the above substances did not cause appreciable error. However, concentrated extract from "vitamin-free" casein contained appreciable amounts of niacin.

The experimental results indicated that the so-called characteristic symptoms of single water soluble vitamin deficiencies, such as chromotrichia, spectacled eye, alopecia, and bloody whiskers were rarely observed in the multiple water soluble vitamin deficient "A" or "C" dietary rats. "A" dietary animals receiving either nicotinic acid, or pyridoxine and "C" animals receiving only pyridoxine developed alopecia. This syndrome was absent in the other animals.

Albino weanling rats ingesting only water soluble vitamin supplements of thiamine, riboflavin, or pantothenic acid with "A" diet had lower mortality rates, better growth curves and better appearance than animals on diets containing only pyridoxine, nicotinic acid, inositol, choline or biotin. Pyridoxine and nicotinic

acid were associated with high mortality and marked loss in weight under the experimental conditions. Although choline supplemented "A" animals had greater comparative weight losses, the mortality was surprisingly low.

Animals on "B" diet plus combinations of water soluble vitamin supplements indicated that pantothenic acid caused a greater weight gain per unit time per rat (40 gms. in 50 days per rat) than the addition of pyridoxine, choline, inositol, or vitamin E to the "B" diet. These experiments also strengthen the view that characteristic single water soluble vitamin deficiency symptoms may develop only when the diet is adequate with respect to the "other" vitamins.

No relationship was found between red and white cell counts and alopecia symptoms as shown in Table II.

TABLE II.

Time Fed		Red Cell	White Cell	
Days	Weight	Count x 10^6	Count x 10^3	Alopecia Symptoms
35	40	8.1	—	—
35	64	8.6	8.0	—
61	70	8.7	10.0	+++
76	50	9.8	6.0	+++
43	100	9.5	12.0	—
50	124	12.2	10.0	—
105	130	8.5	14.2	++++
112	112	11.1	10.0	++
40	—	8.5	8.2	Normal

Analysis of kidney and liver tissue from animals on "A" or "C" diets ingesting supplements of single water soluble vitamins indicated that under the experimental conditions dietary pyridoxine or choline lowered the inositol content in these organs. Dietary niacin in "A" animals lowered kidney and liver tissue pantothenic acid content. Choline supplements to animals on "A" or "C" diets, lowered kidney and liver inositol content, however no alopecia was observed in the latter animals in contrast to the former who showed definite alopecia.

Animals on "C" diet plus single vitamin supplements had less kidney or liver tissue pantothenic acid than the corresponding "A" animals.

Analysis of the cecal contents for the four vitamins showed that those animals on "A" diet receiving no water soluble vitamin supplements or supplements of thiamine, riboflavin, or pantothenic acid had more "cecal" vitamin than those receiving only choline, niacin or biotin. Rats receiving only dietary pyridoxine showed appreciable amounts of cecal vitamin. Animals receiving "C" diet plus single vitamin supplements had less cecal vitamin than corresponding "A" animals.

Nitrogen and sulphur determinations of rat hair from animals on "A" or "C" diets plus single water soluble vitamin supplements showed that pyridoxine, nicotinic acid, or choline lowered the N/S ratio.

The water soluble material from ground hair when incorporated into basal diet "C" at a 5% level caused a noticeable increase in weight in comparison to animals on "C" basal diet, indicating the presence of nutritive substances in the water soluble extract of hair. Rats receiving only dietary pantothenic acid supplements to "A" diet gave higher hair pantothenic acid values than other animals. Properly prepared samples of hydrochloric acid hydrolyzed rat hair gave niacin values in close agreement to values obtained from the same hair which was treated to "ball mill grinding" to increase its solubility. It was shown that the ball mill technique enables one to obtain around 95% of the total hair vitamin or "vitamin-like" content in soluble form.

It was found that the ratios of concentration of riboflavin, pantothenic acid, niacin and inositol in normal rat hair is approximately the same as those existing in normal rat kidney and liver tissue.

Human hair was also studied. Vitamin analyses showed that partially bald men had lower inositol values than normal rat or human hair with the possible exception of human grey hair which had "less than normal" inositol content.

A large amount of the previously mentioned data is tabulated in Table III. Each figure represents an average figure from several animals. Individual values used to obtain the tabulated average figure represent at least duplicate analyses.

CONCLUSIONS

1. Dietary alopecia in rats may involve (a) heredity, (b) vitamin deficiency, (c) water soluble vitamin interrelationships, which as a whole, tend to decrease in the tissue the amount of those water soluble vitamins which are necessary for good hair production.

2. The vitamins which are required to a greater degree in the prolongation of the rat's life when water soluble vitamin deficient diets are fed are (a) thiamine, (b) riboflavin, (c) pantothenic acid. Vitamins which are required to a lesser degree from this viewpoint are (a) nicotinic acid, (b) inositol and (c) pyridoxine.

3. The water soluble vitamins which are required to a greater degree for normal rat hair growth using synthetic diets are (a) inositol, (b) pantothenic acid, (c) thiamine, (d) biotin. From this standpoint, pyridoxine, choline and nicotinic acid are required to a lesser extent.

4. When either pyridoxine or nicotinic acid in large amounts was the only water soluble vitamin added to the basal diet, a greater frequency of alopecia was observed in the animals.

5. Analysis of kidney and liver from rats on water soluble vitamin deficient diets indicate that (a) information regarding the relative requirements of the different types of tissue for a single water soluble vitamin may be determined. (b) Ingestion of large amounts of a single water soluble vitamin may change the concentrations of other vitamins in the tissues of rats. (c) Symptoms of water soluble vitamin deficiencies may be accented or alleviated by ingestion of large amounts of a single vitamin which may not be directly concerned in the specific deficiency symptoms.

TABLE III.

Diet	Supplement	Symptoms at 30 Days	% Gain or Loss in Wt.	Kidney				Liver				Cecum				Water Soluble Vitamin Content** Hair				
Rat Experiments				R*	P	N	I	R	P	N	I	R	P	N	I	R	P	N	I	N/S Ratio
Stock	None	Normal	+210	35	48	136	1900	22	88	150	475	14	10	70	..	3.1	12.0	15.5	195	4.89
"A"	None	No striking symptoms	+16	37	62	140	4500	18	72	157	800	18	19	7	..	.6	.5	8.2	221	2.96
"C"	None	No striking symptoms	..	30	16	...	...	24	61	...	...	1	6	..	28	2.0	.5	7.8	200	3.26
"A"	B_1	Fair condition	+40	20	41	175	200	21	105	165	475	10	10	31	..	..	..	..	..	3.62
"A"	Riboflavin	Pediculosis	+30	41	48	157	700	21	59	145	1500	12	1	11	..	..	..	..	..	2.93
"A"	Pantothenic Acid	Fair condition	+40	45	55	155	1800	26	130	150	3100	6	12	18	..	1.0	10.9	16.0	260	3.74
"A"	Pyridoxine	Alopecia	—30	20	90	136	860	20	78	150	900	40	15	14	..	3.0	2.1	5.0	60	1.83
"A"	Choline	Fair condition	—20	29	50	160	150	29	93	156	670	3	7	16	..	..	..	..	..	2.60
"A"	Nicotinic Acid	Alopecia	—35	25	48	155	2000	15	35	150	600	1	3	20	..	..	..	..	..	2.45
"A"	Biotin	Acrodynia	—10	20	70	168	2200	34	70	130	420	7	9	12	..	..	..	..	..	3.10
"C"	Choline	No striking symptoms	—	30	20	...	1000	20	58	...	640	4	4	..	0	2.0	1.4	19.4	40	2.94
"C"	Pyridoxine	Alopecia	—	29	20	...	820	16	50	...	400	3	3	..	0	3.2	1.0	12.0	100	2.79
"C"	8 Water Sol. Vitamins	None	—	..	..	...	...	..	..	...	...	..	..	..	..	3.8	8.7	18.4	195	4.43
Human Experiments																				
Normal		Blonde hair														1.4	0.5	12.0	197	2.30
Normal		Brown														1.0	0.3	5.0	130	2.41
Normal		Black														1.5	0.3	6.9	150	2.32
Normal		Red														1.2	1.4	6.1	380	2.45
Normal		Grey														0.8	0.6	4.9	37	...
Normal		Alopecia														0.2	0.6	2.2	98	...

*Abbreviation for riboflavin, pantothenic acid, niacin and inositol.

**Kidney and liver tissue vitamin values are expressed in micrograms/gm. on the moist basis. Cecal vitamin values are for total cecal contents. Hair values are on the dry basis.

6. Symptoms, such as alopecia, chromotrichia, weight loss, and general appearance which develop when rats are fed water soluble vitamin deficient diets, are controlled to a certain extent at least by (a) tissue water soluble vitamin interrelationship, (b) cecal bacterial vitamin synthesis, (c) hereditary factors, (d) other factors, such as room temperature, bacterial infection, intestinal absorption, etc.

7. Analysis of gastrointestinal contents from the rat for water soluble vitamins indicate that: (a) pepsin, peptone, albumin and bile salts do not appreciably affect the microbiological bacterial assay method in higher dilutions. (b) Cecal bacterial vitamin synthesis aids in preventing certain vitamin deficiencies, since the bacteria may synthesize more vitamin than they utilize.

8. Nitrogen and sulphur analyses of rat hair from animals on water soluble vitamin deficient diets show that ingestion of large amounts of pyridoxine, choline or nicotinic acid lowers the N/S ratio. This is due to a decrease of the nitrogen in the hair rather than an increase in sulphur. The other water soluble vitamins studied do not show this effect when given as single supplments to the rat using a water soluble vitamin deficient diet.

9. Analysis of rat hair for water soluble vitamins, using a new "grinding" technique, shows (a) the ratios of concentrations of the water soluble vitamins or "vitamin-like substances" are approximately the same as in other tissues of normal animals. (b) Their concentrations are affected by the type of diet fed. (c) Vitamin interrelationships may affect hair vitamin content under certain conditions of diet.

10. Starch contains "unrecognized substances" which from a nutritive role may make it a better source of dietary carbohydrate than glucose.

VITA

Leo Joseph Novak was born in DeKalb, Illinois, June 4, 1914. Received elementary education in Chicago. Attended J. Sterling Morton High School and studied two years at J. Sterling Morton Junior College. Received the A. B. degree from the University of Illinois in 1935. From 1935 to 1942 he was employed in the capacity of an analytical chemist. In 1939 received the M.S. degree from the University of Illinois. The title of this work was "Chemical and Biological Studies of Vitamin B_1 and Related Compounds."

He is married and has one child, a girl. At present he is in charge of chemical analysis and is also serving as a nutritional chemist for Libby, McNeil and Libby. He is a member of the Institute of Food Technologists and Association of Vitamin Chemists.